Microbial implications associated with stomach flushing of Little Penguins

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Abstract
The stomach flushing technique is a vital tool in bird dietary studies. The technique requires a tube to be inserted into the penguin's mouth and passed through the oesophagus to the stomach. General practice does not include cleaning of the tube between penguins. This report investigates if the stomach flushing tube can be a vehicle to transmit potential pathogens from a sick penguin to a healthy penguin, and if implementation of aseptic or disinfection practice is warranted in the stomach flushing technique. A total of 19 tubes from 19 penguins were examined for bacterial presence from May until August 2007. This paper presents new recommendations for stomach flushing procedures from a microbial perspective to ensure that birds subjected to this are not jeopardised by practices that may promote the transfer of potential pathogens from one penguin to another. (*The Victorian Naturalist* **128** (4), 2011, 128–131)

Keywords: *Eudyptula minor,* stomach flushing, microbes, Little Penguins

Introduction

Seabirds are excellent indicators of the health of the marine ecosystem (Barrett *et al.* 2007). For example, the monitoring of seabird diet can provide data on fluctuations in fish populations (Barrett *et al.* 2007). The identification of critical prey items and monitoring of seabird diets are of significant importance in understanding and managing their ecological requirements (Deagle 2007; Gales 2007). Many different methods are used to determine the critical prey items in the diet of seabirds, from direct feeding observations, emetics, the collection of regurgitated pellets, and the observation of stomach samples from carcasses (Barrett *et al.* 2007); however, some of these techniques are considered to be either lethal (Sieburth 1959), highly stressful to the bird or difficult to employ due to the feeding ranges of seabirds. Therefore, in 1984 Wilson described an improved method for stomach flushing of penguins. This technique now has been used extensively to obtain stomach contents from a range of birds (Chiaradia *et al.* 2003; Gales, 1987; Neves *et al.* 2006; Randall and Davidson 1981). The method requires a latex tube to be passed through the oesophagus of a bird to its stomach. Once inserted, water is pumped (either via a water pump or syringe) into the bird's stomach, the bird is inverted and pressure is placed on the bird's stomach to induce regurgitation. This method can be repeated many times until the returning water is clear of regurgitates (Gales 1987; Wilson 1984). This procedure has allowed scientists to acquire more comprehension of the ecological requirements of seabirds (Deagle *et al.* 2007); however, certain limitations were identified by researchers with this technique and modifications made (Chiaradia, *et al.* 2003; Gales 1987; Preston 2008). Limitations identified included a limit on the number of times an individual could be flushed (e.g. maximum of three flushes per penguin), assessment of stomach index (indicates the availability of space in the stomach) and a restriction on the amount of water that could be injected into the penguin's stomach (Preston 2008).

Data obtained at the 6th International Penguin Conference indicated that most penguin biologists (80%, n=10) do not implement a cleaning regime (e.g. disinfection of stomach flushing tube) or aseptic practice (e.g. sterilised tube per penguin) when flushing penguins (pers. obs. Andrea Chiaradia, Knowles Kerry, and Tiana Preston pers. comm.). Penguins have been known to be infected by a range of pathogens (e.g. *Pasteurella multocida* and *Corynebacterium*), which in some cases are responsible for high mortality rates post infection

(DeLisle *et al.* 1990; Leotta *et al.* 2006; Murray and Houston 2005; Williams and Ward 2002). This documentation is of concern as the tube with which the stomach is flushed may act as a vector for cross contamination from penguin to penguin. This study investigated whether bacteria can be transferred from one penguin to another by documenting the presence of bacteria on the tube used on free ranging little penguins. Furthermore, the efficiency of different disinfectants for potential use in the field to clean tubes between animals was tested. This paper also presents new recommendations for the stomach flushing technique from a microbial perspective to ensure that birds subjected to this procedure are not jeopardised by practices that may promote the transfer of bacteria from one penguin to another.

Site and Sampling

Data were collected opportunistically during May (N=7) July (N=7) and August 2007 (N=5) on free ranging penguins as part of a study on the diet of little penguins at the St Kilda Breakwater (Melbourne, Victoria) (Preston 2008). This collaboration allowed us to collect microbial data opportunistically from the stomach flushing tube used on penguins without causing additional stress to the penguins. A total of 19 individual penguins were captured and flushed. The stomach flushing procedure implemented during the dietary study followed Wilson (1984) and included the modifications outlined by Chiaradia *et al.* (2003) with the addition of 1) determination of stomach index, and 2) the replacement of a water pump with 140 ml syringes (Preston 2008). During May, swabs were taken from the tubes used for stomach flushing before the procedure and immediately after the tube was removed from each penguin. There was no cleaning regime implemented during this field trip (i.e. the tube was not cleaned between penguins); however, a cleaning regime (disinfection) was introduced. During the July field trip a 1% aqueous sodium hypochloride solution commonly used to disinfect babies bottles (Milton) was trialled, and in August a commonly used Veterinary disinfectant (F10SC) was trialled on the cleaning tube. The cleaning regimes implemented in this study required the tube used to flush stomachs to be soaked in either the Milton Antibacterial solution or F10SC for five minutes before being used on another penguin (i.e. after completion of stomach flushing). After disinfection, the tube was rinsed internally and externally with distilled water to remove any residue before reuse and to ensure the disinfectant did not cause an impact on natural microflora of these penguins. A swab was then collected from the tube to determine the effectiveness of the disinfection treatment.

Bacterial cultures were grown on Horse Blood, MacConkey, and nutrient agar and incubated at 37°C for 48 hours. Quantification of the total number of bacterial colonies was conducted based on gram stains (May and July) and morphological characteristics (all months). No gram stains were conducted on specimens collected in August. Disinfection in this study was defined as the removal of at least 80% of all colony forming units of all bacterial species.

Results

Presence of bacteria on stomach flushing tube

Fourteen penguins were sampled during the months of May and July. In total, 80 distinctive species of bacteria were obtained from tubes. The mean number of different bacteria found per tube per penguin was 5.5 (S.D. =0.5; N = 14). The mean number of gram negative bacteria found on the tube was higher (May: Mean = 2.3, S.D. =1.25; N =7; July: Mean =2.14; S.D. =0.7 ; N =7) than gram positive species (May: Mean =0.6 ; S.D. =0.53 ; N =7 ; July: Mean =1.14; S.D. =1.07; N =7).

Trial of Disinfectants

Before tube disinfection the mean number of morphologically distinct bacteria found on the tube was 3.4 (S.D. =1.25; N =7) in the month of July, and 5.5 (S.D. = 2.65; N = 5) in the month of August. Cultures from samples collected immediately after disinfection, did not grow any bacteria (Fig. 1). Both Milton and F10SC were 100% effective in removing bacteria from the tubes used for flushing in the field ($F = 84$, $P = <0.01$; $F = 17.286$, $P = <0.001$) respectively (Fig. 1).

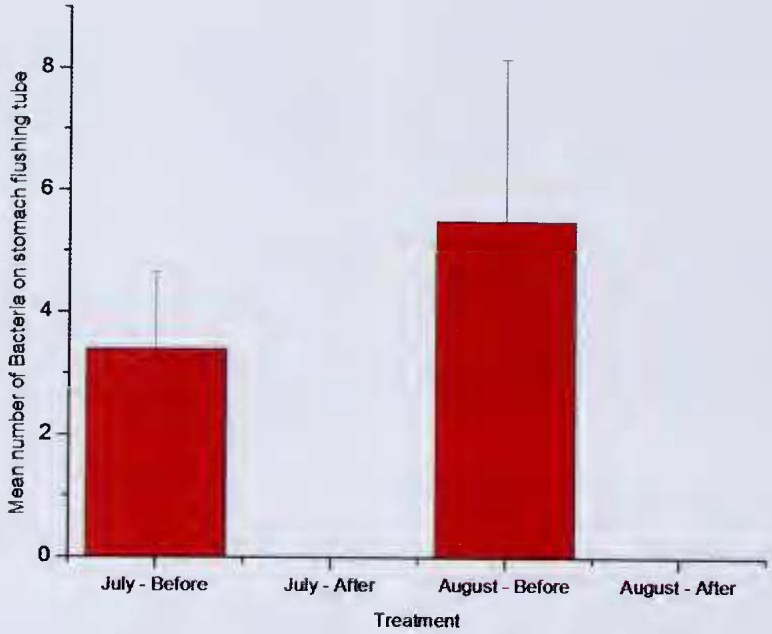

Fig. 1. The mean number of bacteria present on the stomach flushing tube pre- and post cleaning with Milton (July) and F10SC (August). N represents total number of bacteria found on the tube pre-flushing for each month.

Discussion

Penguins are susceptible to infectious diseases (Boerner, *et al.* 2004; Broman, *et al.* 2000; Clarke and Kerry 1993; Clarke and Kerry 1999; Goyache, *et al.* 2003; Leotta, *et al.* 2006; Murray and Houston 2005; Thouzeau, *et al.* 2003; Zdanowski, *et al.* 2004) including Avian Cholera *Pasteurella multocida,* and Avian Diphtheria *Corynebacterium,* which have been responsible for high rates of mortality in penguin and seabird colonies (DeLisle *et al.* 1990; Leotta *et al.* 2006; Murray and Houston 2005; Williams and Ward 2002). Results from this study demonstrate that bacteria adhere to the tube(s) used to flush stomachs and, therefore, have the potential to transmit bacteria from one individual to another when the tube is used multiple times without disinfection. In light of these results, it is suggested that aseptic practice become routine in the technique of stomach flushing. Results demonstrated that the two disinfectants selected were effective at removing bacteria in the field. Furthermore, the two disinfectants were inexpensive, simple to use, time efficient and safe to use in the field without compromising research objectives. Alternatively, tubes could be sterilised (e.g. autoclaved) in the laboratory before fieldwork and each penguin could be flushed using a different tube.

Furthermore, aseptic/disinfection application should not be limited to penguins exclusively, but to any animal subjected to stomach flushing. This could be of fundamental importance to endangered and threatened bird populations that are subjected to this procedure, such as the Yellow-eyed Penguins *Megadyptes antipodes* (Moore and Wakelin, 1997), the Royal Penguin *Eudyptes schlegeli* (Horne 1985), the Southern Rockhopper Penguin *Eudyptes chrysocome chrysocome*, the Northern Rockhopper Penguin *Eudyptes chrysocome moseleyi* (Horne 1985; Ray and Schiavini 2005), Wandering Albatrosses *Diomedea exulans*, (Cooper *et al.* 1992; Xavier

et al. 2003) and the White-chinned Petrel *Procellaria aequinoctialis* (Cooper *et al* 1992) to ensure that these species are not jeopardised by dietary studies that utilise the stomach flushing technique.

Although further analysis needs to be conducted for identification and quantification purposes, the results have demonstrated the presence of bacteria on the tubes used for stomach flushing. Because medical equipment can become contaminated with infectious microorganisms after any procedure, the Therapeutic Goods Administration of Australia (TGA, 2004) states that all medical equipment must be decontaminated before reuse to prevent the transmission of microorganisms from one individual to another. Therefore, as a precautionary measure, researchers should consider either using individually sterilised tubes for each penguin or disinfecting the tube used for stomach flushing between birds.

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